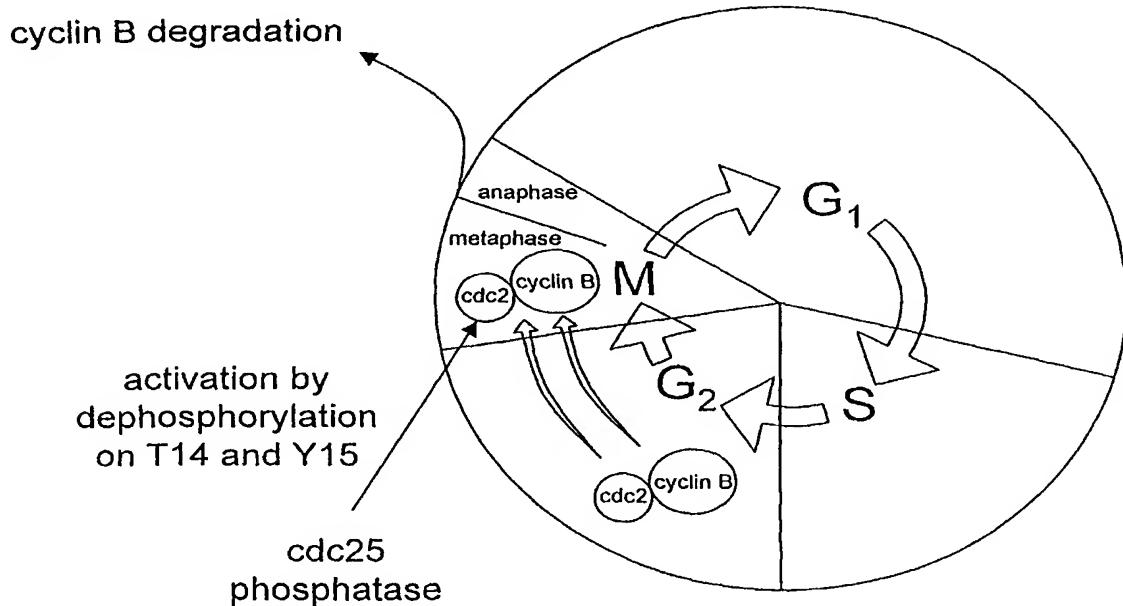




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/00	A2	(11) International Publication Number: WO 99/62503 (43) International Publication Date: 9 December 1999 (09.12.99)
(21) International Application Number: PCT/EP99/03625		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 26 May 1999 (26.05.99)		
(30) Priority Data: 98109845.2 29 May 1998 (29.05.98) EP 99105693.8 19 March 1999 (19.03.99) EP		
(71) Applicant (for all designated States except US): CNRS (CENTRE NATIONAL DE RECHERCHE SCIENTIFIQUE) FRANCE INNOVATION SCIENTIFIQUE ET TRANSFERT [FR/FR]; 135, boulevard Saint Michel, F-75005 Paris (FR).		Published <i>Without international search report and to be republished upon receipt of that report.</i>
(71)(72) Applicant and Inventor: EISENBRAND, Gerhard [DE/DE]; Gustav Kirchhoffstrasse 3, D-69120 Heidelberg (DE).		
(74) Agent: MÜLLER-BORÉ & PARTNER; Graffinger Strasse 2, D-81671 München (DE).		

(54) Title: USE OF INDIGOID BISINDOLE DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT TO INHIBIT CYCLIN DEPENDENT KINASES



(57) Abstract

The present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK 2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

"Use of indigoid bisindole derivatives for the manufacture of a medicament to inhibit cyclin dependent kinases"

Description

The present invention relates to the use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases, particularly CDK 1, CDK2, CDK 4 and CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1).

5

Indigoid bisindoles comprise a spectrum of natural dye stuffs. Many of these can be obtained from plants. Accordingly, indirubine, indigo and isoindigo are natural products which can be obtained from different plants: namely, *Baphicacanthus cusia* (Acanthaceae), *Indigofera suffruticosa* (Fabaceae), *Isatis indigotica* (Brassicaceae) and others. Indican, a glycoside which is found in plants, gives glucose and 3-hydroxyindole due to acidic or enzymatic hydrolysis. 3-Hydroxyindole is converted by air-oxidation into indigo and its isomers. Indigo naturalis (Chinese: quingdai) is the natural blue dye obtained from plant material, e.g. *Isatis indigotica* (Brassicaceae). Indirubine, an isomer of indigo, can be found in Indigo naturalis in an amount of up to 60% (Falbe J. & Regitz M., Römpf Chemie Lexikon (1992), 9. Aufl., Stuttgart, Georg Thieme Verlag). It occurs also in *Isatis tinctoria* in an amount of up to 5% which is indigenous to Central Europe (Gelius R., Z. Chem., 20, (1980), 340-341).

10

15

20

25

Indigo naturalis is reported to be used in traditional Chinese medicine as a hemostatic, antipyretic, anti-inflammatory and sedative agent in the treatment of bacterial and viral infections. Antileukemic effects of Indigo naturalis have also been reported, with indirubine being the effective principle (Ji X. et al., Acta Pharm. Sin., 16, (1981), 146-148; Gan W. J. et al., J. Hematol., 6, (1985), 611-613). Furthermore, derivatives of indirubine are known for a long time as dyes of low persistence.

Few, structurally quite different types of p34^{cdc2} inhibitors have been described up to now. They are either of natural origin or derived from natural compounds and show varying degrees of inhibitory activities. Examples are Staurosporine, an alkaloid from Streptomyces sp, Butyrolactone-I from Aspergillus terreus var., 5 Flavopiridol, a novel promising anti-tumour agent derived by partial synthesis from a parent structure found in the indian plant Dysoxylum binectariferum, 9-Hydroxyellipticin from the plants Ochrosia elliptica and Ochrosia acuminata, the purine derivatives olomoucine, roscovitine and isopentenyladenine and some peptides. The mechanism of these compounds is based on competitive inhibition 10 of ATP binding (Meijer L., Trends in Cell Biology, 6, (1996), 393-397).

For determination of the IC 50-values: p34^{cdc2}/cyclin B was purified from M phase starfish (*Marthasterias glacialis*) oocytes by affinity chromatography on p9^{CKShs1}-Sepharose beads, from which it was eluted by free p9^{CKShs1} as described 15 (Meijer et al., Eur. J. Biochem., 243, (1997), 527-536). It was assayed with 1 mg histone H1 (Sigma type III-S)/ml, in the presence of 15 µM [γ -³²P] ATP (3,000 Ci/mmol; 1mCi/ml) in a final volume of 30 µl. After 10 min. incubation at 30°C, 25 µl aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of Whatman P81 phosphocellulose paper, and after 20 sec., the filters were 20 washed five times (for at least 5 min. each time) in a solution of 10 ml phosphoric acid/liter of water. The wet filters were transferred into 6 ml plastic scintillation vials, 5 ml ACS (Amersham) scintillation fluid was added and the radioactivity measured in a Packard scintillation counter. The kinase activity was expressed in pmoles phosphate incorporated into histone H1/10 min incubation 25 or in % of maximal activity.

The strongest inhibitors of p34^{cdc2} are derivatives of staurosporine (IC₅₀ = 0,003-0,03 µM). The selectivity of these inhibitors is, however, rather poor. They also show more or less potent inhibitory activity to quite a wide range of 30 cellular kinases. Table 1 shows the specificity of known chemical inhibitors of cyclin-dependent kinases (IC₅₀-values given in µM) (cf. Meijer L., Trends in Cell Biology, 6, (1996), 393-397).

Tab. 1:

Enzyme	Staurosporine	UCN-01	Butyrolactone-I	Flavopiridol	Olomoucine	Roscovitine	9-Hydroxy-ellipticine
5	CDK1	0.003-0.009	0.031	0.60	0.40	7	0.65 ^a ca. 1
	CDK2	0.007	0.030	1.50	0.40	7	0.70 ND
	CDK4	< 10.000	0.032	no effect	0.40	>1000	>100 ND
	MAPK	0.020	0.910	94	ND	30	30 ND
	PKA	0.008	ND	260	145	>2000	>1000 ND
	PKG	0.009	ND	ND	6	>2000	>1000 ND
	PKC	0.005	0.007	160	ND	>1000	>100 ND
10	Tyrosine-kinase	0.006 0.006 (Src)	ND	>590 (EGF-R)	25 (EGF-R)	440 (EGF-R)	70 (I-R)

CDK: cyclin-dependent kinase; EGF-R: epidermal growth factor receptor tyrosine kinase; I-R: insulin receptor-tyrosine kinase; MAPK: mitogene-activated protein kinase; ND: not determined; PKA: cAMP-dependent protein kinase;PKG: cGMP-dependent protein kinase.

^aIC₅₀-value for racemic mixture; IC₅₀ for (R)-roscovitin is 0.45 μM.

Cyclins and cyclin dependent kinases (CDK) have an essential role for driving the cell through the cell cycle (cell division cycle, cdc). During the cell division cycle, oscillations in concentrations and activities of cyclins are observed. This applies, e.g. to cyclins D and E in the so-called G1-phase of the cell cycle and to cyclinA (S- and M-Phase) and cyclinB (G2- and M-Phase).

The cyclin dependent kinases are activated by association with a member of the cyclin-family. Up to now, eight human CDKs have been described: CDK1 (= p34^{cdc2}), CDK2 to CDK8. CDK-proteins consist of a catalytic subunit and a regulatory subunit, the cyclins (Meijer et al., Eur. J. Biochem., 243, (1997), 527-536). Every step of the cell division cycle is regulated by specific CDK/cyclin complexes which ascertain a strict control. Important checkpoints are at the transition from G1 phase to S phase and from G2 phase to M phase (Pines J.,

Cancer Biology, 6, (1995), 63-72). The p34^{cdc2}/cyclinB complexes are important components at the G₂-M-checkpoint.

Fig.1 shows the points of action of the cyclin CDK complex cdc2/cyclinB in the
5 cell division cycle (M = mitosis, cell division; G = gap; S = synthesis;
interphase = G₁ + S + G₂).

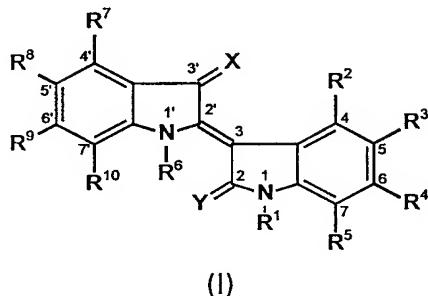
The cdc2/cyclinB complexes are the primary active protein kinases in mitosis.
They accumulate in an inactive state in the cytoplasm in interphase cells, and
10 are then rapidly activated by cdc25 phosphatase and translocated into the
nucleus at the beginning of mitosis (Pines J., Hunter J., Cell Biol., 115(1),
(1991), 1-17). CyclinB is degraded at the metaphase-anaphase transition,
inactivating cdc2, which is necessary for exit from mitosis (Glotzer et al.,
Nature, 349(6305), (1991), 132-138; Murray A.W., Nature, 339(6222), (1989),
15 280-286; Surana U. et al., Cell, 65(1), (1991), 145-161). Multiple changes of
CDK proteins and their regulators are associated with the development of
human tumours (Cordon-Cardo C., Am. J. Pathol., 147(3), (1995), 545-560).

Thus, the technical problem underlying the present invention is to provide new
20 inhibitors for cyclin dependent kinases, particularly CDK 1, CDK2, CDK 4 and
CDK 5, more particularly ATP:Proteinphosphotransferase p34^{cdc2} (CDK1), which
exhibit a high selectivity as well as high efficiency compared to the inhibitors
known in the art.

25 The solution to the above technical problem is achieved by the embodiments
characterized in the claims.

In particular, the present invention relates to the use of indigoid bisindole
derivatives for the manufacture of a medicament for inhibiting cyclin dependent
30 kinases, particularly CDK 1, CDK2, CDK 4 and CDK 5, more particularly
ATP:Proteinphosphotransferase p34^{cdc2} (CDK1), in mammals, preferably in man.
Preferably, the indigoid bisindole derivatives are selected from indigo
derivatives, isoindigo derivatives or indirubine derivatives.

In a preferred embodiment of the present invention, the indirubine derivate is a compound having the general formula (I)



5

wherein the groups R¹ and R⁶ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a straight-chain or branched-chain alkyloxy group having 1 to 18 carbon atoms; a straight-chain or branched-chain methylenealkoxy group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can

10

15

20

25

30

comprise one or more heteroatoms; or an acyl group; a methylene-amino group -CH₂-NR¹¹R¹², wherein R¹¹ and R¹² have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or a methylenesulfonate group; R², R³, R⁴, R⁵, R⁷, R⁸, R⁹ and R¹⁰ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an alkoxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a substituted or unsubstituted methylenaryloxy group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatom(s); a -CONR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonate group; a sulfonamide group; a

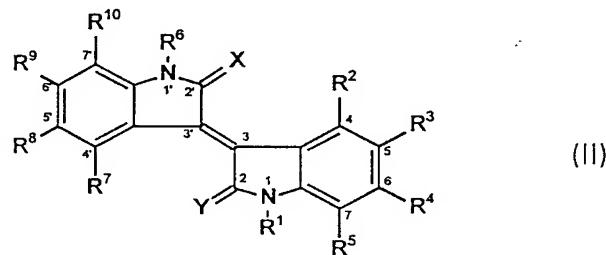
-SO₂NR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; an azo group -N=N-R¹³, in which R¹³ represents an aromatic system which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or R¹ and R⁵, and R⁶ and R¹⁰, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH₂ groups; and X and Y can be the same or different and represent an oxygen atom; a sulfur atom; a selenium atom; a tellurium atom; a NR¹⁴ group in which the group R¹⁴ represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an aralkyl group, or a sulfonate group; or a NOR¹⁴ group, wherein the group R¹⁴ has the above definitions.

15

With respect to the benzene nuclei constituting the indirubine derivates of the the above general formula (I) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the indirubine system. Furthermore, the indirubine derivatives having the above general formula (I) can also be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.

20

In another embodiment of the present invention, the isoindigo derivate is a compound having the general formula (II)



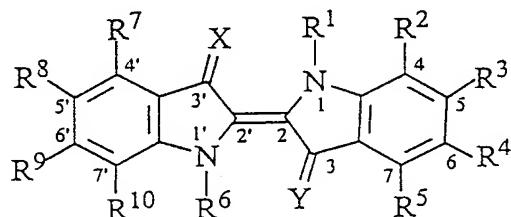
wherein R¹ to R¹⁴ and X and Y have the above definitions.

5 With respect to the benzene nuclei constituting the isoindigo derivates of the above general formula (II) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the isoindigo system. Furthermore, the isoindigo derivatives having the above general formula (II) can be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.

10

In a further embodiment of the present invention, the indigo derivate is a compound having the general formula (III)

15



20

(III)

wherein R¹ to R¹⁴ and X and Y have the above definitions.

25 With respect to the benzene nuclei constituting the indigo derivates of the above general formula (III) one or more ring atoms can be replaced by nitrogen atoms. Further, one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, can be condensed to the indigo system. Furthermore, the indigo derivatives having the above general formula (III) can be bound to a polyethyleneglycolester or a polyethyleneglycoether by ester bondings or ether bondings, respectively.

30

The above indigoid bisindole derivatives having the general formulas (I), (II) or

(III) can also be in the form of their physiologically acceptable salts.

In the search for new selective inhibitors of cellular signalling pathways, it has surprisingly been found that indigoid bisindole derivatives are highly selective 5 inhibitors of the enzyme complex p34^{cdc2}/cyclinB. An inhibition of p34^{cdc2}-kinase by indigoid bisindole derivatives is not described in the prior art. Surprisingly, it has turned out that indigoid bisindole derivatives are both highly selective and highly effective inhibitors of cdc2-kinase and other cyclin dependent kinases 10 (CDK's), showing IC₅₀-values with the isolated enzyme down to submicromolar range. The inhibition of CDK-activities also is observed in cell culture, using human tumor cell lines. As an example, indirubin-3'-monoxime was found to inhibit histone H1 phosphorylation as a measure for CDK1/cyclin B activity after 24 h incubation of MCF-7 mammary carcinoma cells. Moreover, the content of cyclin B complex was significantly reduced. Cells arrested by serum deprivation 15 after treatment for 24 h with indirubin-3'-monoxime in serum containing medium exhibited an arrest in G1-phase of the cell cycle at low micromolar concentrations of the substance. In concentrations ≥ 5 µM, an additional arrest at G2/M-phase of the cell cycle became apparent. Cells arrested in G2/m by Nocodazole treatment after release of the block exhibited a significant increase 20 of G2/M arrested cells under treatment with ≥ 5 µM indirubin-3'-monoxime, resulting in a massive accumulation of cells in G2/M. Concomitantly with the observed intracellular effects, growth inhibition was induced in the same concentration range, resulting in an IC₅₀-value of 3.3 ± 0.7 µM after 3 days incubation (IC₅₀ = concentration that induces 50% growth inhibition as compared to vehicle control). In addition, induction of apoptotic cell death was 25 observed.

Based on the above mentioned selective inhibitory potency down to the nanomolar range, indirubine derivatives can be used for analytical biochemistry, 30 especially for the study of cell cycle effects. Furthermore, these compounds can be used for the treatment of diseases in patients, which are connected to the loss of proliferation control without any restriction to these potential areas of application. These include cancers, psoriasis, cardiovascular diseases

(stenosis, restenosis) (Brooks et al., J. Biol. Chem., 272, (1997), 29207-2921-1), infectious diseases (unicellular parasites (Trypanosoma, Toxoplasma, Plasmodium, etc.), fungi, etc.), nephrology (glomerulonephritis: Pippin et al., J. Clin. Invest., 100, (1997), 2512-2520), neurodegenerative disorders such as 5 Alzheimer disease (Imahori, K., Uchida T., J. Biochem., 121, (1997), 179-188), viral infections such as cytomegalovirus (Bresnahan W. A. et al., Virology 231, (1997), 239-247) and HIV (Mancebo H.S.Y. et al., Genes & Dev., 11, (1997), 2633-2644).

10 The present invention is explained further by the following examples:

Example 1: Synthesis of indirubine

To a solution of 0.42 g (2.4 mmol) of indoxyl acetate in 20 ml methanol under 15 argon 0.35 g (2.4 mmol) of isatin and 0.55 g (5.2 mmol) of sodium carbonate are added. The mixture is stirred for 30 min at ambient temperature. After 24 h standing at ambient temperature, the reaction mixture is filtered off. The precipitate is washed with little methanol and water until the filtrate shows a neutral pH. Residual water is removed by storage in an evacuated exsiccator 20 over potassium hydroxide. Recrystallisation from ethanol or pyridine gives deep purple crystals (Russell G.A., Kaupp G. (1969), J. Am. Chem. Soc., 91, 3851-9, modified).

Yield: 0.51 g (81%), fine, deep-purple needles, Fp: 341-343 °C

CHN-analysis: ($C_{16}H_{10}N_2O_2$); MW: 262,26 g/mol; calc.: 73.3% C, 3.8% H, 25 10.7% N; found: 73.2% C, 4.0% H, 10.6% N

mass spectrum: m/z = 262: (M⁺, 100%), 234: (43%), 205 (25%), 158 (3%), 131 (4%), 103 (7%), 76 (3%)

¹H-NMR and ¹³C-NMR-spectrum are in accordance with the proposed structure.

IR-spectrum: 3340 cm⁻¹: ν (N-H), 1710 cm⁻¹: ν (3'-C=O), 1650 cm⁻¹: ν (2-C=O), 1590 cm⁻¹: ν (C=C, aryl), 1450 cm⁻¹: ν (C=C, aryl), 745 cm⁻¹: ν (aryl 30 with four neighbouring H-atoms).

UV/Vis-spectrum (DMSO): 290 nm, 363 nm, 383 nm (shoulder), 551nm

Essentially the same synthetic procedere was applied for the following Examples 2 to 9 and 11 and 12:

Example 2: 5-Iodoindirubine

5 Yield: 80%, fine, deep-purple needles, Fp: 334-335 °C (decomposition);
CHN-analysis ($C_{16}H_9IN_2O_2$); MG = 388.16 g/mol; calc.: 49.5% C, 2.3% H,
7.2% N; found.: 49.7% C, 2.5% H, 7.1% N;
Mass spectrum: 388 (M^+ , 100%), 360 (3%), 269 (9%), 261 (6%), 233
(16%), 205 (16%), 128 (1%);
10 1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.
UV/Vis-spectrum (DMSO): 370 nm, 386 nm (shoulder), 555 nm.

Example 3: 5-Bromoindirubine

Yield: 70%, fine, deep-purple needles;
15 CHN-analysis ($C_{16}H_9BrN_2O_2$); MG = 341.16 g/mol, calc.: 56.3% C, 2.7% H,
8.2% N; found 56.4% C, 2.7% H, 8.2% N;
Mass spectrum: 342(M^+ , 100%), 340 (M^+ , 99%), 314 (18%), 262 (64%),
233 (34%), 205 (81%), 177 (10%);
1 H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

20

Example 4: 5-Chloroindirubine

Yield: 95%, fine, deep-purple needles;
CHN-analysis ($C_{16}H_9ClN_2O_2$); MG = 296.70 g/mol; calc.: 49.5% C, 2.3% H,
7.2% N; found: 49.7% C, 2.5% H, 7.1% N;
25 Mass spectrum: m/z = 296 (M^+ , 100%), 268 (39%), 239 (8%), 233 (35%),
205 (50%), 177 (7%), 153 (6%), 137 (7%), 77 (7%), 120 (4%), 102 (6%),
77 (7%).
 1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

30

Example 5: 5-Fluoroindirubine

Yield: 92%, fine, deep-purple needles;
CHN-analysis ($C_{16}H_9FN_2O_2$), MG = 280.25 g/mol, calc.: 68.6% C, 3.2% H,
9.9% N; found: 68.0% C, 3.2% H, 9.9% N;

Mass spectrum: m/z = 281 ($M^+ + H^+$, 19%), 280 (M^+ , 100%), 252 (73%), 223 (32%), 176 (6%), 140 (7%), 121 (13%), 94 (4%), 76 (12%), 77 (7%), 57 (4%), 44(15%).

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

5

Example 6: 5-Methylindirubine:

Yield: 92%, fine, deep-purple needles;

CHN-analysis ($C_{17}H_{12}N_2O_2$), MG = 276.28 g/mol, calc.: 73.9% C, 4.4% H, 10.1% N; found: 73.8% C, 4.3% H, 10.2% N;

10 Mass spectrum: m/z = 276 (M^+ , 100%), 261 (10%), 248 (47%), 247 (53%), 220 (6%), 219 (18%), 205 (7%), 171 (4%), 165 (10%), 138 (4%), 133 (15%), 104 (7%), 77 (7%);

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

15 Example 7: 5-Nitroindirubine

Yield: 88%, fine, deep-purple needles;

CHN-analysis ($C_{16}H_9N_3O_4$), MG = 307.26 g/mol; calc.: 62.5% C, 3.0% H, 13.7% N; found: 62.4% C, 3.0% H, 13.3% N;

20 Mass spectrum: m/z = 307 (M^+ , 5%), 276 (10%), 262 (100%), 234 (23%), 205 (22%), 158 (6%), 131 (10), 104 (19%), 76 (12%), 50 (6%).

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 8: 5'-Bromoindirubine

Yield: 92%, fine, deep-purple needles;

25 CHN-analysis ($C_{16}H_9BrN_2O_2$), MG = 341.16 g/mol, calc.: 56.3% C, 2.7% H, 8.2% N; found: 55.7% C, 2.5% H, 8.0% N.

1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Example 9: 5,5'-Dibromoindirubine

30 Yield: 94%, fine, deep-purple needles;

CHN-analysis ($C_{16}H_8Br_2N_2O_2$), MG = 420.06 g/mol; calc.: 45.7% C, 1.9% H, 6.7% N; found: 45.8% C, 2.0% H, 6.4% N.

1H -NMR-spectrum is in accordance with the proposed structure.

Example 10: Indirubine-3'-oxime

5 Indirubine-3'-oxime was synthesized by reaction of indirubine with hydroxylamine hydrochloride in a pyridine solution (Farbwerke vorm. Meister Lucius & Brüning in Hoechst a.M., Patentschrift des Reichspatentamtes Nr. 283726 (1913)). ^{13}C -NMR-spectroscopy revealed the location of the hydroxyimino residue in 3'-Position ($\delta(\text{C}2) = 171.05 \text{ ppm}$; $\delta(\text{C}3') = 145.42 \text{ ppm}$; DMSO-d₆, RT)

10 Yield: 90 %, red crystals;

CHN-analysis ($\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_2$), MG = 277.30g/mol; calc.: 69.3% C, 4.0% H, 15.2 % N; found: 69.0% C, 4.0% H, 14.9% N;

15 ^1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

15 Example 11: Indirubine-5-sulfonic acid

Yield: 76%, crystalline, deep-purple substance;

Mass spectrum: 388 (M⁺, 100%), 360 (3%), 269 (9%), 261 (6%), 233 (16%), 205 (16%), 128 (1%).

20 ^1H -NMR- and ^{13}C -NMR-spectrum are in accordance with the proposed structure.

Table 2 shows the structures of the compounds of Examples 1 to 11.

Tab. 2:

Example **compound** **R¹** **R²** **R³** **X**

Example	compound	R ¹	R ²	R ³	X
<u>1</u>	Indirubine	H	H	H	O
<u>2</u>	5-Iodoindirubine	I	H	H	O
<u>3</u>	5-Bromoindirubine	Br	H	H	O
<u>4</u>	5-Chloroindirubine	Cl	H	H	O
<u>5</u>	5-Fluoroindirubine	F	H	H	O
<u>6</u>	5-Methylindirubine	CH ₃	H	H	O
<u>7</u>	5-Nitroindirubine	NO ₂	H	H	O
<u>8</u>	5'-Bromoindirubine	H	Br	H	O
<u>9</u>	5,5'-Dibromoindirubine	Br	Br	H	O
<u>10</u>	Indirubine-3'-oxime	H	H	H	NOH
<u>11</u>	Indirubine-5-sulfonic acid	SO ₃ H	H	H	O

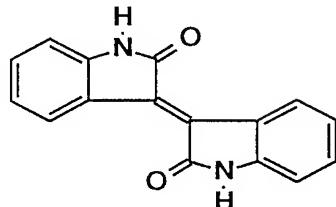
Table 3 shows the specificity of the compounds of Examples 1 to 11 (IC₅₀-values given in μ M) to inhibit cdc2 kinase in comparison to other cellular kinases.

Tab. 3:

Example	cdc2	cdc25	PKA	PKC
<u>1</u>	2	150	no Effect	no Effect
<u>2</u>	0.3	130	no Effect	no Effect
<u>3</u>	0.35	32	ND	ND
<u>4</u>	0.40	55	ND	ND
<u>5</u>	2.0	70	ND	ND
<u>6</u>	0.8	60	ND	ND
<u>7</u>	2.2	60	ND	ND
<u>8</u>	6.5	ND	ND	ND
<u>9</u>	7	ND	ND	ND
<u>10</u>	0.18	ND	ND	ND
<u>11</u>	0.055	110	ND	ND

ND: not detected; PKA: cAMP-dependent protein kinase;
PKC: Ca^{2+} - dependent protein kinase

Example 12: Isoindigo



Isoindigo was synthesized by reaction of oxindole with isatin in acetic acid with addition af hydrochloric acid (Wahl A., Bayard P., Comptes Rendues Hebdomadaires des Seances de L'Academie des Sciences, 148, (1909), 716-719).

10 Yield: 84%, crystalline, brown substance;
CHN-analysis ($\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_2$), MG = 262.26 g/mol; calc.: 73.3% C, 3.8% H, 10,7% N; found: 73.0% C, 3.8% H, 10.9% N;
Mass spectrum: m/z = 262 (M^+ , 100%), 234 (85%), 220 (5%), 205 (18%), 190 (4%), 177 (5%), 151 (5%), 132 (17%), 103 (6%), 76 (4%), 32 (26%).
15 $^{1}\text{H-NMR}$ - and $^{13}\text{C-NMR}$ -spectrum are in accordance with the proposed structure.

Isoindigo shows an IC_{50} -value of $80\mu\text{M}$ for the $\text{p34}^{\text{cdc2}}/\text{cyclinB}$ complex.

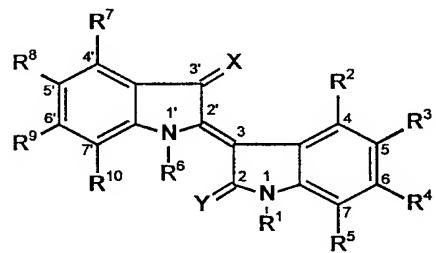
20 Table 4 shows the kinase inhibition selectivity of indigo, indirubin, 5-chloroindirubine, indirubine-3'-monoxim and indirubine-5 sulfonic acid. The indicated IC_{50} values were calculated from respective dose response curves and are presented in μM .

Tab. 4:

	Enzyme	indigo	indirubine	5-chloro-indirubine	indirubine-3'-monoxime	indirubine-5-sulfonic acid
5	CDK1/ cyclin B	> 1000.000	10.000	0.400	0.180	0.055
	CDK2/ cyclin A	70.000	2.200	0.750	0.440	0.035
10	CDK2/ cyclin E	> 1000.000	7.500	0.550	0.250	0.150
	CDK4/ cyclin D1	> 100.000	12.000	6.500	n.t.	0.300
	CDK5/ p35	> 100.000	5.500	0.800	0.100	0.065

Claims

1. Use of indigoid bisindole derivatives for the manufacture of a medicament for inhibiting cyclin dependent kinases.
2. Use according to claim 1, wherein the cyclin dependent kinases are selected from CDK 1, CDK 2, CDK 4 or CDK5.
3. Use according to claim 1 or 2, wherein the indogoid bisindole derivatives are selected from indigo derivatives, isoindigo derivatives or indirubine derivatives.
4. Use according to claim 3, wherein the indirubine derivate is a compound having the general formula (I)



(I)

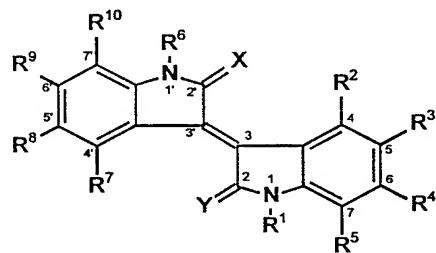
wherein the groups R¹ and R⁶ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a methylenehydroxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms; a straight-chain or branched-chain alkyloxy group having 1 to 18 carbon atoms; a straight-chain or branched-chain methylenealkoxy group having 1 to 18 carbon atoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a substituted

or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms; a mono-, di- or trialkylsilyl group having 1 to 6 carbon atoms independently of each other in each instance in the straight-chain or branched-chain alkyl group; a mono-, di- or triarylsilyl group with substituted or unsubstituted aryl groups independently of each other in each instance; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; or an acyl group; a methyleneamino group -CH₂-NR¹¹R¹², wherein R¹¹ and R¹² have the above definitions; a benzyl group, wherein the benzene nucleus can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a physiological amino acid residue bound to the nitrogen as an amide; an O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or a methylenesulfonate group; R², R³, R⁴, R⁵, R⁷, R⁸, R⁹ and R¹⁰ can be the same or different and represent a hydrogen atom; a halogen atom; a hydroxy group; a nitroso group; a nitro group; an alkoxy group; a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups; a substituted or unsubstituted aryl group which can comprise one or more heteroatoms; a substituted or unsubstituted aralkyl group which can comprise one or more heteroatoms; a substituted or unsubstituted aryloxy group which can comprise one or more heteroatoms;

group which can comprise one or more heteroatoms; a substituted or unsubstituted methylenearyloxy group which can comprise one or more heteroatoms; a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a methylenecycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatoms; a trifluoromethyl group; a -COM group; a -COOM group; a -CH₂COOM group, wherein M is hydrogen, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, or an aryl group which can comprise one or more heteroatoms and can be substituted with one or more halogen atoms, one or more alkyl groups or one or more alkoxy groups; a -NR¹¹R¹² group, wherein R¹¹ and R¹² can be the same or different and represent a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or wherein the nitrogen atom is part of a cycloalkyl group having 3 to 7 carbon atoms which can comprise one or more heteroatom(s); a -CONR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; a hydroxylamino group; a phosphate group; a phosphonate group; a sulfate group; a sulfonate group; a sulfonamide group; a -SO₂NR¹¹R¹² group, wherein R¹¹ and R¹² have the above definitions; an azo group -N=N-R¹³, in which R¹³ represents an aromatic system which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups; or a O-glycoside or a N-glycoside, wherein the glycoside is selected from monosaccharides or disaccharides; or R¹ and R⁵, and R⁶ and R¹⁰, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH₂ groups; and X and Y can be the same or different and represent an oxygen atom; a sulfur atom; a selenium atom; a tellurium atom; a NR¹⁴ group in which the group R¹⁴ represents a hydrogen atom, a straight-chain or branched-chain alkyl group having 1 to 18 carbon atoms which can be substituted by one or more carboxyl groups, phosphoryl groups or sulfonate groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, an

aralkyl group, or a sulfonate group; or a NOR¹⁴ group, wherein the group R¹⁴ has the above definitions.

- 5. Use according to claim 4, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (I) are replaced by nitrogen atoms.
- 10. Use according to claim 4 or 5, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the indirubine system.
- 15. Use according to anyone of claims 4 to 6, wherein the compound having the general formula (I) is bound to a polyethyleneglycolester or a polyethyleneglycoether.
- 8. Use according to claim 3, wherein the isoindigo derivate is a compound having the general formula (II)



(II)

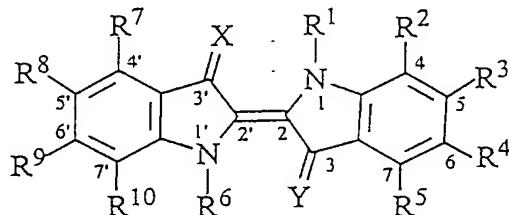
- 20. wherein R¹ to R¹⁴ and X and Y have the meanings as defined in claim 4.
- 25. Use according to claim 8, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (II) are replaced by nitrogen atoms.

10. Use according to claim 8 or 9, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the isoindigo system.

5 11. Use according to anyone of claims 8 to 10, wherein the compound having the general formula (II) is bound to a polyethyleneglycolester or a polyethyleneglycoether.

10 12. Use according to claim 3, wherein the indigo derivate is a compound having the general formula (III)

15



(III)

20 wherein R¹ to R¹⁴ and X and Y have the meanings as defined in claim 4.

13. Use according to claim 12, wherein one or more ring atoms of the benzene nuclei of the compound having the general formula (III) are replaced by nitrogen atoms.

25

14. Use according to claim 12 or 13, wherein one or more aromatic or non-aromatic ring systems which can comprise one or more heteroatoms independently of each other, are condensed to the indigo system.

30

15. Use according to anyone of claims 12 to 14, wherein the compound having the general formula (III) is bound to a polyethyleneglycolester or a polyethyleneglycoether.

16. Use according to anyone of claims 1 to 15, wherein the indigoid bisindole derivative is in the form of a physiologically acceptable salt.

17. Compound which is 5-fluoroindirubine.

5

18. Compound which is 5-nitroindirubine.

19. Compound which is 5,5'-dibromoindirubine.

